REMARKS

1. Discussion of Amendments

1.1. We have amended claim 1 in several respects:

(1) to reconcile the "recombinant arylsulfatase A" terminology in the preamble with the "polypeptide" having arylsulfatase A activity language in the body.

(2) to reconcile the "concentrating, purifying and formulating language" at the beginning of step ii with the "purification process" language later in step ii.

(3) to specify that in the "passive step" contemplated by step ii, there is no binding of the polypeptide, and

(4) to require that the continuous culturing is for at least one week.

(5) to clarify the term "production".

With regard to point (1), the preamble uses the term "arylsulfatase A". It is evident from the specification and original claims that this term was intended to encompass, not only the wild-type human ASA of SEQ ID NO:2, but also various enzymatically equivalent molecules. Compare, e.g., original claims 1 and 2. And amended claim 1 clearly is intended to still cover those polypeptides that have ASA activity and comprise a sequence at least 95% identical to SEQ ID NO:2, even if they are not identical to SEQ ID NO:2. However, inconsistently, original claims 3-6 recited "arylsulfatase A or its equivalent", which could be read as implying that the term "arylsulfatase A" standing alone refers only to SEQ ID NO:2.

To avoid any future confusion as to the meaning of "arylsulfatase A" or "rASA" in the claims, claim 1 has been

amended so that the preamble refers to a "recombinant polypeptide with arylsulfatase A (ASA) activity", and the body further limits the nature of that polypeptide by virtue of the preexisting "wherein" clause. Conforming amendments have been made to the dependent claim.

With regard to the "concentrating, purifying and formulating" language of step (ii), the remainder of that step describes a purification process and does not describe "formulating". Moreover, the preamble recited the production of a polypeptide, not the production of a pharmaceutical formulation. Additionally, in the summarization of the invention at P12, L35 - P13, L7, it is stated that "the process ... **may** comprise one or more of the following general steps ... A. Culturing ... B. Concentration... and purification... C. Formulation...." The "may" indicates that "formulating" is not an essential step of the invention. This is confirmed at P19, L24-26, stating that "one could for instance omit the formulation step as well as the filling and freeze-drying if the aim is not to provide a final product suitable to medicinal use."

Hence recitation of "formulating" in step (ii) of claim 1 appeared undesirable; we have removed it from claim 1, but added a new claim 67 that recites an additional formulating step. For related reasons, we have removed steps VII and VIII from claim 11 but added new claims 68 and 69 that recite them.

Insofar as "concentrating" versus "purifying" is concerned, it appears to us that the definition of "purifying" at P8, L16-19 is quite broad, and clearly encompasses "concentrating" as used in the specification. Page 11, line 1 refers to "concentration from the supernatant" and implies that it is effectively the first purification step of the "purification process" of claim 1 , step (ii). P19, L4-6 state that step (I) (cp. claim 12) of the more broadly defined step (ii)(cp. claim 1) is "concentration by tangential flow

filtration".

In general, we would say that a "concentration" step is one whose goal is simply the reduction of the volume of liquid medium (i.e, of water) , see P20, L9-13.

P19, L28 specifically states that this step (I) may be omitted, and we note that it is not recited in claim 11. Thus, the recitation of "concentrating" appeared superfluous in the context of claim 1, and has been withdrawn.

Hence, we have amended claim 11 to refer simply to the "process of step (ii)" rather than to the "concentration and purification process" of that step. Note that concentration is still recited in claim 12.

With regard to the "passive step" limitation, there is basis at P24, L37-38: "The polishing step is thus initiated at pH 6.0 where the enzyme **will not bind** to a first affinity chromatography resin or a first cation exchanger (passive step)".

With regard to the time period limitation, this limitation is imported from prior claim 42, and finds basis at P17, L3-4. New claims 60-62, with more stringent temporal limitations, have the same basis.

Finally, claim 1 spoke of the cells producing the polypeptide in the liquid medium. Strictly speaking, a precursor polypeptide is expressed, the signal peptide is cleaved off, and the mature polypeptide is secreted into the culture medium. See P2, L12-16 and P16, L27-28. We amended claim 1 to use the more precise terminology.

Since it is the secreted mature polypeptide that is purified, and must have the ASA activity, we slightly revised the preamble and the wherein clause to reflect this.

Conforming amendments have been made to the dependent claims.

New claims 63-66 find basis as follows:

63 ("wherein said continuous culturing is carried out in a cell culture system comprising one or more bioreactors, said cells being in said bioreactor(s), said system comprising means for collecting medium comprising said polypeptide, and a cell retention device for retaining cells in said bioreactor(s) when said medium is collected, and said system comprising means for adding fresh medium."): P16, L30-35.

64 ("wherein said cell retention device is at least 95% efficient."): P16, L32 and P55, fifth parameter.

65 ("wherein medium comprising said polypeptide is collected, while retaining cells in said bioreactor(s), at least once prior to the conclusion of said culturing."): as for claim 63, and also compare the preferred period of culturing per P17, L1-4 with the preferred frequency of collecting medium, see P55, L1-3.

66 ("in which medium comprising said polypeptide is collected at least daily."): P55, L1-3.

New claims 67-69 are based, as previously explained, on prior claims 1 and 11.

1.2. There is a sentence fragment at P24, L18-19. The intent was to provide the characteristics of small (non-industrial) scale purifications, as distinct from the large (industrial) scale purifications alluded to at L13-18. The sentence has been completed accordingly. In this regard, note that P43, L38 identifies 200-400 ml as "small to medium size" and P44, L1 says that "large scale" is greater than 2L.

2. Response to Objections

Since claim 11 no longer recites steps (VII) and (VIII), it doesn't need a conjunction between them. However, we have amended claim 11 to place a conjunction between steps (V) and (VI).

3. Prior Art Issues

Claims 1, 8-9, 11-16 and 42-49 stand rejected as anticipated by Fogh, WO 02/098455. We traverse.

We previously argued that the claims are not anticipated because:

(1) The method of Fogh is a batch process and the presently claimed method is a continuous process.

(2) Fogh does not disclose a "polishing" step including a "passive" step, wherein the arylsulfatase A passes (i.e., without retention and subsequent elution) through a cation exchange chromatography resin or membrane and/or affinity chromatography resin.

3.1. With regard to the distinction (1) above, the examiner responds that Fogh et al. discloses "a small-scale and semi-large scale fermentation, both of which are cultured continuously for 163 hours, or about a week".

Apparently, the examiner interprets the term "continuous culturing" according to the lay meaning of each word taken individually, i.e., meaning culturing without interruption. So interpreted, the term would not be particularly limiting unless coupled with a minimum culturing period.

Claim 1 has been amended to recite, "and wherein the continuous culturing is for a period of at least one week[1]". Basis for this amendment appears at P17, L3-4. We note that a week is 7*24=168 hours, which is more than the 163 hours referred to by the examiner. Hence, even if the reference taught continuous culture, culture for only 163 hours would not anticipate.

Additionally, new claims 60 to 62 recite continuous culturing for at least 2, 3 and 4 weeks, respectively. Basis is at P17, L3-4.

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[1] The "one week" limitation was previously recited in claim 42, now cancelled as superfluous.

We are of the opinion that the term "continuous culturing" has a special meaning in the art. In particular, it is generally contrasted with the term "batch culturing". It is clear that we consider our continuous culturing to be distinct from batch culturing given that we describe our culturing system at P16, L30-35, and at P17, L1 we comment, "One immediate advantage of this system as compared to a **batch** system is to allow for an effective production phase extending over longer time." [emphasis added]

Generally speaking, a batch system is one in which at the start, all the starting materials (reagents and catalysts in a chemical system, media and cells in a biological system) are loaded in, and at the end (and only at the end), the contents of the reactor are completely removed. There are no inputs or outputs in-between.

In the case of batch culturing, that necessarily means that over the course of the batch culture, nutrients are depleted from the medium, and that in term limits the length of the effective production phase.

Thus, at a minimum, "continuous culturing" must be different from batch culturing; it must feature "intermediate" inputs and/or outputs, i.e., the introduction of fresh media, or withdrawal of product, after the start and before the culturing comes to an end by virtue of the removal of the cells (whether or not those cells are subsequently transferred to a new reactor).

We are presently reviewing the use of the term "continuous culture" in the literature and will address this usage in due course. In the meantime we have added claims 63-66:

> 63 (New). The method of claim 1 wherein said continuous culturing is carried out in a cell culture system comprising one or more bioreactors, said cells being in said bioreactor(s), said system comprising means for collecting medium comprising

said polypeptide, and a cell retention device for retaining cells in said bioreactor(s) when said medium is collected, and said system comprising means for adding fresh medium.

64 (New). The method of claim 63 wherein said cell retention device is at least 95% efficient.

65 (New). The method of claim 63 wherein medium comprising said polypeptide is collected, while retaining cells in said bioreactor(s), at least once prior to the conclusion of said culturing.

66 (New). The method of claim 65 in which medium comprising said polypeptide is collected at least daily.

It is possible that some of the limitations of claims 63-66 are inherent in the proper definition of "continuously culturing" and hence superfluous, but given the examiner's interpretation of the term, we thought it best to present these claims at this time. They clearly distinguish the cited reference.

We assume that the examiner's citation of "small scale" fermentation is a reference to Fogh Example 4 ("small-scale cultivation of producer cell") and the reference to "semi-large scale fermentation" is to Fogh Example 5 ("semi-large-scale cultivation").

Example 4 (P36, L9) refers to incubation of cells in a humidified incubator for 72-96 hours (3-4 days) followed by inoculation of shake flasks and harvesting 80-100 hours post-inoculation. The cells are separated from supernatant (containing the ASA) by centrifugation resuspended in fresh medium, and passaged to new shake flash cultures. (P36, L17-24).

The first incubation must be considered a batch culture

process, since the cells are subcultured to a new culture vessel (the shake flask). It is not continuous culture because there is no withdrawal of old medium or supply of fresh medium while essentially retaining the cells. Also, the culture is for less than one week.

The second cultivation, in the shake flasks, is likewise a batch culture process since the cells are completely separated from their medium, and placed in new shake flash cultures. This culture is likewise less than one week.

In continuous culture, medium containing the desired secretion products is withdrawn, and fresh medium added, without removing all of the cells from the cell culture system or separating all of the medium from the cells. See P16, L32-35. See also P17, L21-22, P55, L1-6. Note that the disclosed embodiment had a "CRE" (cell retention efficiency) above 95% (P55, table under "Separation Parameters"); this term is explained at P56, L1-3.

Referring next to prior art Example 5, this is explicitly characterized as being a batch process, see e.g. P37, L25 ("2 batches were produced"). Each batch was cultivated, as stated by the Examiner, for 163 hours (Tables 4 and 5).

Example 5 is distinguished in that the present claim requires continuous culturing for at least one week (168 hours), whereas Example 5 teaches mere batch culturing for 163 hours.

3.2. The examiner also argues that Fogh "Example 6 is for a large production, not purifying the combined 'batches' of the small scale and semi-large scale product".

With regard to the scale of the purification, for the sake of accuracy we point out that the process of example 6 (while intended for scale up) is actually run on a 20-200 ml scale (see Fogh, P40, L3-4). Thus, P40, L32-33 says that "spinner produced media was concentrated... to 100 ml". This is clearly small scale. Note that at P43, L38, we characterize 200-400 ml as small to medium size, and that in Table 5, our

purification was of 7990 ml.

However, we are not sure of the relevance of the scale as there is no cultivation or purification scale limitation in any of the rejected claims.

3.3. With regard to distinction (2) above, the examiner contends that "the claims recite that a passive step is passage (loading and eluting) through a cation exchange chromatography resin or membrane, which Fogh et al. discloses on page 42".

The Examiner's contention is clearly inconsistent with the previously cited P18, L29-P19,L2.

> Furthermore it is preferred that the concentration and purification process of (ii) comprises a polishing step including a passive step, wherein the arylsulfatase A passes through an affinity chromatography resin or membrane and/or a cation chromatography resin or membrane, and an active step, wherein the arylsulfatase A is detained within and subsequently eluted from an anion exchange membrane or resin. This combination of passive and active steps is suggested from the surprising finding that whereas most contaminating proteins binds to an anion exchange matrix at pH values less than 5.8, preferably at pH values around 5.5-5.7, arylsulfatase A will pass the cation exchange matrix and subsequently bind to an anion exchange resin. It is believed that a change in the structure of the enzyme from a dimer to an octamer at pH values below 5.8 is responsible for this surprising effect. This change in structure has physiological relevance since the enzyme is active in the lysosomes at low pH.
> [emphasis added]

It is clear that in a "passive step", the ASA "passes through" the membrane i.e., moves through it without retention, and thus without need for subsequent elution". What the examiner calls "loading" is clearly what the quoted passage equates with "detained within", and thus is part of an

active step.

We would be completely justified to rely on the present "passive step" language of claim 1. However, to expedite prosecution, we have amended claim 1 to make explicit an inherent feature of the passive step, i.e., that the ASA is not bound. See P24, L37-38: "The polishing step is thus initiated at pH 6.0 where the enzyme will not bind to a first affinity chromatography resin or a first cation exchanger (passive step)".

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By: _____
Iver P. Cooper
Reg. No. 28,005

624 Ninth Street, N.W.
Washington, D.C. 20001
Telephone: (202) 628-5197
Facsimile: (202) 737-3528
IPC:lms
G:\ipc\n-q\Plou\Fogh5A\fogh5a.ptoamendaftfinal.wpd